prevents the expected reduction in collateral flow after PCI of a stenotic vessel. There is a direct dose-response relation between exercise capacity gained and coronary collateral flow augmentation in the angiographically normal vessel.

1045-191 High-Density Lipoprotein-Induced Tube Formation Requires the Activation of Ras/Raf/Mitogen-Activated Protein Kinase in Human Coronary Artery Endothelial Cells

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Objectives: High density lipoprotein (HDL) levels have been shown to be inversely correlated with coronary artery disease, but the mechanisms of the direct protective effect of HDL on endothelial cells (ECs) are not fully understood. In this study, we investigated the role of the HDL–mediated promotion of angiogenesis in human coronary artery ECs (HCECs).
Methods and Results: We developed an in vitro model of HCEC tube formation on a matrix gel. HDL induced tube formation, in which the dose-response showed that the maximum effective dose of HDL was 100 μg/ml. We also examined the effect of sphingosine-1-phosphate (S1P), which is a carrier of bioactive lipids of HDL, to analyze tube formation. Since HDL contains S1P (about 180 pmol/mg protein), 100 μg/ml of HDL contains about 0.018 μM of S1P. Although we observed 0.02 μM of S1P significantly induced tube formation, it was only 20 % of 100 μg/ml HDL-induced tube formation. HDL-induced tube formation may be partly mediated by S1P. PD98059, an inhibitor of p42/44 mitogen-activated protein kinase (MAPK) activity, but not SB203580, an inhibitor of p38 MAPK activity, suppressed HDL-induced tube formation. Dominant-negative Ras N17 inhibited HDL-induced tube formation. HDL activated Ras by ras pull-down assay and its effect was inhibited by pertusis toxin (PTX). Moreover, HDL activated phospho(p)-p42/44 MAPK, while Ras N17 blocked HDL-induced pp42/44 MAPK.
Conclusions: These results indicate that HDL induced a potent signal through a Ras/MAPK pathway mediated by PTX-sensitive G-protein coupled receptor to the angiogenic phenotype in HCECs.

1045-192 Monocytes, but Not Neutrophils or Lymphocytes Are Essential Mediators of Arteriogenesis

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Background: Blood vessel growth following arterial occlusion is mediated by infiltrating leukocytes. While all three leukocyte subpopulations (neutrophils, lymphocytes, monocytes) are known to play an important role in angiogenesis, only monocytes have been shown to influence the growth of collateral arteries (arteriogenesis). In this study we examined the importance of neutrophils and lymphocytes in a rabbit hindlimb model of arteriogenesis. **Methods:** 36 Rabbits received either Interleukin-8 (IL-8), Neutrophil-activating-protein-2 (NAP-2) or Lymphotactin (Ltn) via osmotic minipumps directly into the collateral circulation after femoral artery ligation. NAP-2 is a relatively selective activator of neutrophils, Ltn chemoattracts lymphocytes and Il-8 has an effect on both celltypes. PBS and MCP-1 treated groups served as controls. After one week, leukocytes around growing collateral arteries were quantified via immunohistology and effects on integrin markers of leukocytes activation (Mac-1, LFA-1) were assessed by flow-cytometry. Collateral conductance was assessed using fluorescent microspheres. **Results**: Although a significant increase in neutrophil accumulation after IL-8 and NAP-2 treatment was detected in-vivo (cells/mm^2: PBS:8,33±2,87,MCP-1:25,23±10,81,IL-8:36,06±12,65,NAP-2:20,67±7,41,Ltn:8,27±5,44) and Il-8 and NAP-2 resulted in neutrophil activation in flow-cytometry (Mac-1 Expression: PBS:21,12±2,11,MCP-1:24,11±2,64,IL-8:32,65±2,10,NAP-2:27,30±2,25, Ltn:17,30±2,16), no significant increase in collateral conductance was observed. Ltn treatment resulted in lymphocyte accumulation (cells/mm^2: PBS:2,48±1,24,MCP-1:4,26±1,16,IL-8:6,88±5,28,NAP-2:2,70±0,57,Ltn:12,80±3,50), but not in collateral artery growth. Collateral conductance: [ml/min/100mmHg]: PBS:50,70±5,15, MCP-1:339,60±19,6, IL-8:58,91±5,56, NAP-2:66,83±8,72, Ltn:52,80±5,37. **Conclusion:** While lymphocytes and neutrophils are known to participate in angiogenesis, their importance for arteriogenesis seems to be neglectible. These findings support the hypothesis of monocytes/macrophages as key mediators of collateral artery growth.

1045-193 Granulocyte-Colony Stimulating Factor Mobilizes and Activates Endothelial Progenitor Cells in Patients With Coronary Artery Disease

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Background: Circulating endothelial progenitor cells may function to repair cardiovascular injury, but are reduced in patients with coronary artery disease. Granulocyte-colony stimulating factor (G-CSF) mobilizes hematopoietic stem cells (CD34+) in healthy subjects, but whether this cytokine mobilizes endothelial progenitor cells capable of endothelial maturation in coronary artery disease patients is unknown. **Methods:** G-CSF (10 mcg/kg) was administered daily for 5 days to 12 coronary artery disease patients, and circulating CD34+ cells, endothelial progenitor cells (CD133/ VEGFR-2+), and mature endothelial cells [CD144 (VE-cadherin), CD31 (PECAM), CD51/61 (alpha$_v$beta$_{III}$ integrin)] were measured by flow cytometry. **Results:** G-CSF increased CD34+ cells from <1 cell/microL of blood at baseline to 60±18 cells/microL within 24 hours of the last dose (p<0.001), similar to CD34+ mobilization achieved in 28 healthy donors >40 years of age at our hospital (78±8 cells/microL). Endothelial progenitor cells were also mobilized, although to low levels (from <1 cell/microL at baseline to 6±3 cells/microL within 24 hours of last G-CSF dose, p<0.001). Mature circulating endothelial cells also increased post-G-CSF: CD34/CD144+ from 16±16 to 107±90 cells/microL, CD34/CD31+ from 54±28 to 224±60 cells/microL, CD34/CD51/61 from 14±14 to 81±64 cells/microL (all p<0.05 vs baseline). One week following completion of treatment, CD34+ cells and endothelial progenitor cells had returned to baseline, but levels of mature endothelial cells remained increased over baseline (p<0.05). **Conclusion:** These findings establish that G-CSF administration to coronary artery disease patients mobilizes CD34+ cells, which includes the endothelial progenitor cell subset, into the circulation. Mobilization is associated with increased cells expressing mature endothelial markers, which persist even at one week following the last dose of G-CSF. This suggests sustained G-CSF-stimulated differentiation along an endothelial cell lineage, with potential therapeutic implications for neovascularization of ischemic myocardium.

1045-194 Impaired Arteriogenic Response to Acute Hindlimb Ischemia in CD8 Knockout Mice

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Background: CD8$^+$ cytotoxic T lymphocytes regulate cellular responses of the immune system, which play a pivotal role in modulating collateral vessel development.
The aim of our study was to investigate if the absence of circulating CD8$^+$ T-cells impairs collateral development after femoral artery ligation in CD8 -/- mice.
Methods and Results: After surgical excision of the femoral artery, Laser Doppler Perfusion Imaging demonstrated reduced collateral flow induction in CD8 -/- mice compared to control mice (C57BL/6J) at day 3 (0.21±0.01 vs 0.29±0.03, p< 0.01) which persisted to day 28 (0.69±0.04 vs 0.90±0.04, p< 0.01). In CD8 -/- mice , when compared to controls, the biological importance of the reduced collateral flow was evident by diminished recovery of hindlimb function (ambulatory impairment score: 1.73 ± 0.18 vs 0.86 ± 0.19, p< 0.01), greater calf muscle atrophy (mean fiber area 767 ± 68 vs 1067 ± 69, μm^2, p< 0.01), and increased fibrotic tissue content (14 ± 1% vs 7 ± 1%, p< 0.01). Exogenous CD8$^+$ T-cells, when infused into CD8-/- mice immediately after ischemia induction, selectively home at the site of collateral formation and, over time, significantly increased collateral flow, improved hindlimb functional recovery, and reduced muscle atrophy/fibrosis.
Conclusions: These results demonstrate that CD8+ T-cells are a critical component of the immune system in regulating the early phase of normal collateral development. CD8 -/- mice demonstrated both delayed and impaired blood flow recovery after femoral artery ligation, and infusion of CD8$^+$ T cells immediately after surgery rescued the phenotype. Our study provides further evidence that the immune system is critical in modulating collateral development in response to peripheral ischemia.

1045-195 Monocyte Chemoattractant Protein-1 Activates Vascular Endothelial Growth Factor- and Tumor Necrosis Factor-Alpha-Mediated Angiogenesis in Ischemic Hindlimbs of Mice

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Recently, we and others have suggested that macrophage accumulation plays a role in angiogenesis in hindlimb ischemia model. Macrophage chemoattractant protein (MCP)-1 is a key molecule to trigger inflammatory changes in various diseases. Thus, we sought to determine the role of the endogenous MCP-1 in ischemia-induced angiogenesis. At day 0, unilateral hindlimb ischemia was induced by excising surgically entire left femoral artery and vein in mice. Immediately after operation, plasmid DNA encoding 7ND, a dominant negative mutant of MCP-1, or the empty plasmid (as control) was injected into the ipsilateral thigh adductor muscle. Serial laser Doppler blood flow analysis showed an abrupt decrease in blood flow, followed by a remarkable recovery, in ischemic hindlimbs of controls. Control mice showed well-developed collateral vessels and capillary formation as assessed by postmortem angiography and immunohistostaining for CD31, respectively, at day 21 after induction of ischemia. In 7ND-treated mice, although the extent of the early decrease in laser Doppler blood flow was similar to that in controls, the recovery was impaired. At day 3, macrophage infiltration and inductions of vascular endothelial growth factor (VEGF) and tumor necrosis factor (TNF)-alpha, known angiogenic factors, were prominent in the adductor muscle of ischemic hindlimbs in controls. 7ND treatment significantly reduced the number of infiltrated macrophages and repressed VEGF and TNF-alpha inductions in response to ischemia at day 3. Moreover, the number of angiographically visible collateral vessels and the capillary density were significantly decreased in ischemic hindlimbs of 7ND-treated mice at day 21. In conclusion, MCP-1-mediated macrophage accumulation may play an important role in ischemia-induced angiogenesis at least in part by activating induction of angiogenic factors such as VEGF and TNF-alpha in ischemic hindlimbs.

1045-196 Fiber Type-Specific Angiogenic Dysregulation in a Genetic Mouse Model of Heart Failure

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Introduction. Chronic heart failure (CHF) leads to intrinsic skeletal muscle abnormalities including slow-oxidative to fast-glycolytic fiber type switching, decreased capillary density, and reduced mitochondrial function. These skeletal muscle abnormalities contribute to clinical exercise intolerance. **Methods**. A genetic mouse model of heart failure induced through cardiac-targeted overexpression of the sarcoplasmic reticulum Ca^{2+} storage protein calsequestrin (CSQ) has been recently characterized. Skeletal muscle (plantaris) from CSQ/CHF mice and wild type (WT) mice was analyzed with triple color immunofluorescence using antibodies specific for myosin heavy chain I, IIa, IIb, and endothelial cells. **Results.** A decrease in oxidative myofibers (I + IIa), a concurrent increase in glyco-

lytic myofibers (IIb + IIx), and an overall decrease in capillary density was observed in CSQ/CHF mice compared to WT littermates. Analysis of fiber type-specific capillary density revealed an increase in type IIa myofiber capillary density and a concomitant decrease in type IIb + IIx myofiber capillary density in the CSQ/CHF mice. **Conclusion**. These results suggest that chronic heart failure causes skeletal muscle fiber type switching and dysregulation of angiogenesis in a fiber type-specific manner. Furthermore, these results support the use of the CSQ/CHF mouse model to further study the role of skeletal muscle maladaptation in chronic heart failure and to explore potential therapeutic targets.

Fiber Type-Specific Angiogenic Dysregulation

	WT	CHF
Fiber Type	%	%
I	6.8	0.6
IIa	56.1	39.9
IIb+IIx	37.1	59.5
Capillary Density	Fibers/Cap	Fibers/Cap
IIa	1.66	2.43
IIb+IIx	0.73	0.47

1045-197 **Evidence for Hypoxia and Hypoxia-Inducible Factor-1-Alpha Mediated Vascular Endothelial Growth Factor Expression in Lipid-Rich Plaque Macrophages as Link Between Inflammation and Angiogenesis**

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Background: Macrophages expressing the pro-angiogenic factor VEGF can contribute to plaque progression and vulnerability via induction of intimal neovascularization. Hypoxia Inducible Factor 1-alpha (HIF-1 alpha) has been identified as key transcriptional regulator of cellular VEGF-synthesis and mediates the angiogenic response of tissues to hypoxia. However, little is known about the pattern of expression of HIF-1 alpha in atherosclerosis. Our objective was to assess in parallel the expression of HIF-1 alpha and VEGF in human and experimental atherosclerosis comparing lipid-rich with fibrous plaque.
Methods: In human atheroma (n=12) HIF-1 alpha and VEGF mRNA and protein expression were evaluated by in situ hybridization and immunohistochemistry (data presented as mean±SEM). In NZW-rabbits (n=14) atherosclerotic lesions were induced by aortic double balloon injury and 9 months of high-cholesterol (HC) diet. Five rabbits were sacrificed at 9 months and were used as baseline (BL). The remaining rabbits were then randomized into continued HC diet (n=5) and normal chow diet (ND) (n=4) for another 6 months. Total serum cholesterol at the end of ND treatment was 27±10 mg/dl vs. 861±143 mg/dl at BL (P<.01) and 526±108 mg/dl with continued HC-diet at 15 months (P<.01).
Results: In human atheroma, HIF-1 alpha and VEGF mRNA and protein expression were increased in lipid-rich compared to fibrous plaque (23±5 % vs. 5±2 %, P<.01; 32±6 % vs. 8±3 %, P<.05) and correlated with high macrophage and neovessel content (P<.05). Double-labeling demonstrated co-localization of HIF-1 alpha and VEGF in inflammatory cells surrounding intimal neovessels in close proximity to cholesterol clefts. In plaques of rabbits with continued HC diet and high serum lipid levels expression of both HIF-1 alpha and VEGF-protein as assessed by immunohistochemistry and Western Blot were significantly increased compared to BL and ND.
Conclusions: The selective detection of HIF-1 alpha in VEGF-expressing macrophages located in lipid-rich plaque tissue points to hypoxia as a possible link between inflammation and angiogenesis. Both processes might synergistically contribute to plaque progression and vulnerability.

1045-198 **Influence of Diabetes Mellitus on Coronary Collateral Flow: A Definite Answer to a Rather Elderly Controversy**

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Background: The influence of diabetes mellitus (DM) on coronary collateral flow is controversial. This may be due to the use of inaccurate means of measuring collateral flow (i.e. angiographic assessment), another reason relates to differences in parameters influencing collateral flow between diabetic and nondiabetic patients (serum lipids, severity of coronary artery disease (CAD)). The purpose of this study was to determine the influence of DM on coronary collateral flow in a large population with variable degrees of CAD using accurate means of collateral flow measurement.
Methods: 187 patients (age 63 +/- 9 years; 86 diabetic and 101 nondiabetic patients) were included in the study. Coronary collateral flow was assessed in 196 stenotic and in 38 angiographically normal vessels using a pressure guide wire (n=145), Doppler guide wire (n=53) or both (n=36) to calculate pressure- or flow-velocity-derived collateral flow index (CFI). Diabetic patients were matched for gender, age, serum lipids and percent diameter stenosis of the vessel undergoing CFI measurement with a nondiabetic control group.
Results: The two groups were exactly balanced for clinical and angiographic data. There was no difference between CFI in the diabetic versus the non-diabetic patients (0.205 ± 0.119 and 0.217 ± 0.128; p=NS). There was still no difference in CFI when only angiographically normal vessels (0.193 ± 0.084 and 0.201 ± 0.072; p=NS) or chronic total coronary occlusions (0.314 ± 0.151 and 0.323 ± 0.186; p=NS) were compared. There was a trend towards a lower number of patients having angina pectoris during the one-minute vessel occlusion in the diabetic group (p = 0.1). There was no correlation between the CFI and the level of HbA1c (as marker for blood glucose control over the last 2-3 months).
Conclusion: There is no difference in coronary collateral flow between diabetic and non-diabetic patients. There is no inverse "dose-response" relation between blood glucose control (represented by the HbA1c level) and coronary collateral flow. There is a trend that diabetic patients have less angina pectoris during a one minute balloon occlusion than non-diabetic patients.

POSTER SESSION

1046

Aortic, Renal, and Peripheral Arterial Diseases

Sunday, March 07, 2004, 3:00 p.m.-5:00 p.m.
Morial Convention Center, Hall G
Presentation Hour: 3:00 p.m.-4:00 p.m.

1046-165 **Measurement of Resting Ankle Brachial Index May Not Accurately Identify Disease in Patients With Signs or Symptoms of Peripheral Arterial Disease**

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Background: The ankle-brachial index (ABI) is useful for objective assessment of lower extremity arterial perfusion, correlating with symptoms and functional severity of peripheral arterial disease (PAD) in population-based studies. This simple, inexpensive measurement can be performed in the primary care office for detection of PAD, and is a marker of increased cardiovascular risk in both symptomatic and asymptomatic patients. The diagnostic accuracy of the resting ABI in patients with symptoms or signs of PAD, however, has not been determined. **Methods:** In order to determine the added diagnostic utility of measuring ABI and pulse volume waveforms (PVR) at rest and after exercise, we analyzed the results of 142 consecutive patients who were referred to the vascular laboratory by *non-vascular* specialists between February and September 2003. **Results:** The average patient age was 68 years; 54% were male. Resting ABI was normal in 44%, abnormal in 38% and not diagnostic due to arterial calcification in 17%. Forty percent were abnormal in those with claudication, 45% in those with ulceration and 28% in those with rest pain. Thirty-six (57%) patients with a normal ABI underwent treadmill exercise testing and 28% of these were found to have an abnormal exercise study indicating the presence of PAD. Among the 24 patients (17%) with calcified vessels, 5 (21%) had abnormal PVR consistent with PAD. **Conclusion:** In symptomatic patients with normal resting ABI, PAD was diagnosed frequently by measuring ABI after exercise. Of the 17% in whom arterial calcification precluded accurate ABI measurement, PVR disclosed PAD in 21%. In patients with suspected PAD, referral to a vascular laboratory for exercise testing and PVR offers more precise diagnosis than office measurements of ABI.

1046-166 **Is the Application of Collagen-Based Vascular Closure Devices After Percutaneous Coronary Interventions Safe and Effective? Intermediate Results From the Prospective Bad Berka Registry**

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Background: Several vascular closure devices (VCD) were developed as an alternative for manual compression after percutaneous interventions (PCI) of the coronary arteries to reduce the time to discharge. We report about the results of the collagen based VCD VasoSeal®ES™ (Datascope Corp.) [VS] and Angio-Seal® (St. Jude Medical) [AS].
Methods: From 10/01 to 09/02 patients with collagen based VCD after percutaneous interventions were enrolled in our prospective registry. During the intervention heparin, aspirin and if necessary clopidogrel were administered. The two VCD were used according to a standardized method, the choice of the device after PCI was left to the discretion of the investigator. Secondary haemorrhage, haematomas (> 5 cm diameter), pseudoaneurysms and AV fistulae were noted as complications. A clinical examination and a Duplex examination in addition was performed routinely at every patient.
Results: 5717 cardiac catheterisations were done until deadline 31.09.2002.
In 1170 cases a VCD was used after coronary intervention, 700 times AS and 470 times VS. One of the above described complications appeared at 54 of 700 cases (7.71%) in the AS group, in the VS group in 35 of 470 cases (7.44 %.) The predominant number of the complications fell on minor complications such as haematoma and smaller secondary haemorrhages (49 of 54 at AS group (7%) and 30 of 35 at VS group (6,4%). Only 5 of 54 (0,7%) of AS and in 5 of 35 (1%) of VS developed more serious complications. One patient in the AS group developed an AV fistula, in four cases we saw pseudoaneurysms. 5 cases of the VS group developed pseudoaneurysms. All aneurysms could be successfully compressed in an ultrasound guided procedure. In one patient of the VS group the